Primer

Plant hormones
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Plant hormones have been studied for more than a century because they have been heavily implicated in the control of plant growth and development. For many years, they have been considered to fall into five classes: auxins, cytokinins, gibberellins, abscisic acid and ethylene. More recently, a variety of additional classes such as brassinosteroids have been added and it seems likely that more will follow.

It is difficult to produce a watertight definition of a plant hormone, and in this context some find the use of the word ‘hormone’ confusing, as any analogy with animal hormones is superficial. Although plant hormones co-ordinate plant growth and development, they do not carry specific signals, from specific tissues, to specific tissues. Perhaps a better analogy would be to compare plant hormones to animal nervous systems, which collect information from a variety of souces, process it and direct appropriate responses. Because of the nature of their role, it is not helpful to produce a list of events in which plant hormones have been implicated. This would be like providing a list of the many physiological and behavioural responses to, for example, hunger in animals. Rather than provide such a list, this article attempts to provide a conceptual framework for plant hormone biology.

Plant hormones as signals

Most plants are autotrophic. Their survival depends on their ability to gather relatively simple substances from the environment: light, carbon dioxide, water, nitrogen and minerals. To achieve this, plants interact with the environment over a massive surface area. Most of this surface area is in the roots and leaves, which continue to develop throughout the life-cycle of the plant. Because development is continuous, the genetic programmes of a plant’s development run against a background of three important variables: the environmental conditions in which it is growing, its physiological status and its developmental stage. The life of the plant is governed by the integration of genetic information with these three variables.

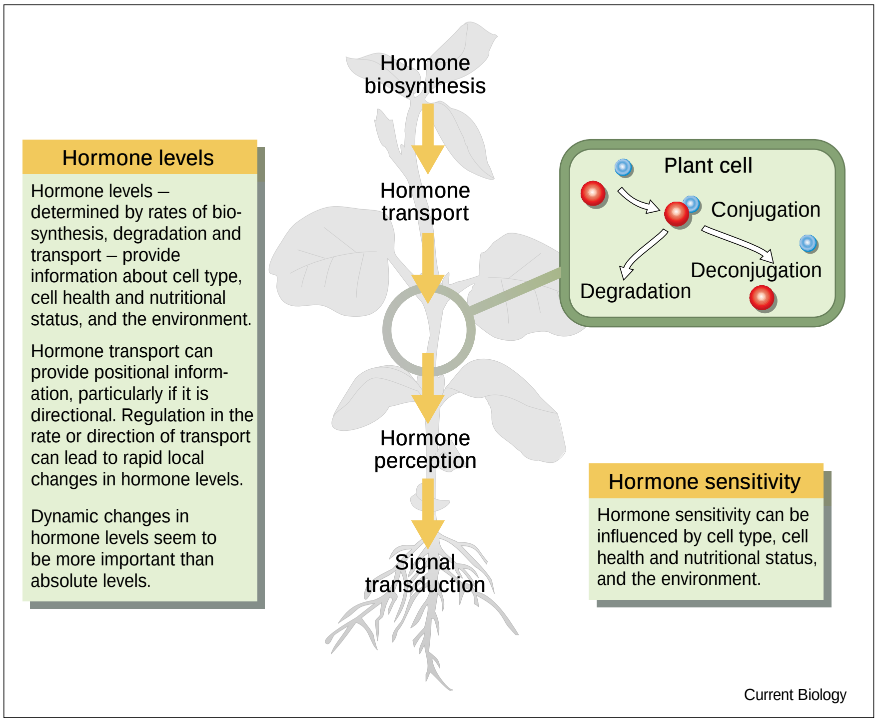
There is a very straightforward relationship between some of these variables, so that information may move directly from one to the other. For example, high light intensity may be reflected in high sucrose levels. High sucrose levels may be reflected in high growth rates. However, there are also more indirect ways of integrating information which involve dedicated signalling molecules. The use of signal molecules that do not have metabolic functions allows a more subtle and sophisticated level of control. For example it may be advantageous that high sucrose levels result in export to a storage tissue rather than high growth rates. One way to achieve this is through a dedicated signalling system.

Plant hormones act as dedicated signalling molecules (Figure 1). A striking feature of plant hormones is that they are closely related to mainstream cellular metabolites. Auxin is similar to tryptophan, cytokinins are related to purine bases, ethylene is only two steps away from *S*-adenosyl methionine, and so on. In the light of this observation, it is tempting to speculate that plant hormones may have evolved from more direct, metabolite-mediated signalling systems.

Defining plant hormones

A large amount of journal space has been taken up with attempts to define the nature of plant hormones,

Figure 1



Plant hormones co-ordinate information from many sources through many points of regulation from biosynthesis to signal transduction.

and often the debate has been rather semantic. A somewhat more interesting question is how can a hormonal signal be distinguished from other mechanisms of information transfer? One answer is that a plant hormone is a substance whose primary function is to carry information. This is obviously a very difficult property to establish and so a more operational definition is required. Several have been proposed including the following five criteria suggested by Elliot Meyerowitz (personal communication). A plant hormone must: act at very low concentrations, less than 10^{-6} M; be synthesized by the plant; be transported some distance, at least one cell diameter; have important and specific effects on physiology, growth or development *in vivo*; act by non-covalent binding to a specific receptor, and remain non-covalently bound, and not covalently modified while acting.

The Meyerowitz criteria are formulated largely to try to exclude environmental factors such as light, and nutritional factors such as vitamins and sucrose. These could easily be mistaken for hormones because of their profound effects on plant development. The case of sucrose is particularly interesting. It does not seem appropriate to consider sucrose to be a hormone, although it has many hormone-like properties. Sucrose is synthesized and transported around the plant, and as the product of photosynthesis its levels reflect environmental and physiological conditions. Its transfer from source to sink tissues can clearly influence patterns of growth and development. There is good evidence that some of the effects of glucose — the breakdown product of sucrose — do not result directly from its use as an energy or carbon source. Indeed, almost the only reasons for considering sucrose not to be a hormone are that it also has important metabolic functions and it seems to act only at millimolar concentrations.

Apart from excluding metabolites and vitamins, the Meyerowitz criteria were also formulated to point out how little we know about the classical hormones — few of them are known to fit all of the criteria. One of the main reasons why plant hormone research has remained such a hotbed of controversy for so long is that we simply have not had sufficient information to begin to understand in any detail how hormones operate. The role of plant hormones in linking many interrelated variables makes for a degree of complexity that is extremely difficult to unravel (for example, see green box about auxin). Three main approaches have been widely used.

Spray and pray

A vast body of literature exists describing experiments involving the effects of the exogenous addition of hormones — for example, by spraying — to different plant species, tissues, developmental stages, and so on. These experiments frequently use unphysiologically high levels of hormone, applied in unphysiological situations such as tissue culture. They do not establish the *in vivo* role of hormones. Nevertheless, a few general conclusions can be drawn from experiments of this type. First, they yield hormone dose–response curves that can be very complex. They are usually log-linear, with low levels and high levels having opposite effects, or with peaks of activity elicited by several different concentrations. Second, different species, tissues, developmental stages, and so on, may respond very differently to the same concentration of hormone. These data led to the suggestion that changes in hormone level are not relevant, but instead plant growth and development are controlled by changes in hormone sensitivity.

Grind and find

Almost as much literature exists describing experiments in which endogenous hormone levels are measured in an attempt to correlate *in vivo* developmental events with changes in hormone concentrations. In some cases good correlations were found, in others there was apparently no correlation at all. The low concentrations in which plant hormones are found may satisfy the first of the Meyerowitz criteria, but they have made it extremely difficult to elucidate hormone biosynthetic pathways. This is compounded by the fact that most hormones seem to be synthesized and degraded via many routes. Hormone transport and sub-cellular localization have been equally difficult to follow, so that local variation in hormone levels cannot easily be measured. This further fuelled the debate about the relative importance of changes in hormone levels versus hormone sensitivity.

Unfortunately, classical biochemical approaches to the study of hormone perception and signal transduction have achieved only limited success. Attention has focused on the isolation of hormone-binding proteins that might act as receptors. This work has produced a few promising candidates but no Rosetta stones. It is obviously rather difficult to resolve a debate about the relative importance of regulating hormone levels versus regulating hormone sensitivity when it is not known how either of these is achieved.

Grow and show

A major problem with the exogenous addition and endogenous levels experiments is the difficulty in establishing causative links between hormone and response *in vivo*. This difficulty is ameliorated by examining the phenotype conferred by single-gene mutations that specifically affect hormone levels, transport or sensitivity. A similar result can be obtained using specific inhibitors. Until quite recently, mutants and/or inhibitors were available in only a relatively small number of cases; however, their use in combination with exogenous

Approaches to the study of auxin action

Spray and pray

Exogenous addition experiments have implicated auxin in tropic growth, and the control of branching and elongation. At the cellular level, auxin influences cell division and elongation and can trigger specific differentiation events. Auxin application can rapidly activate the transcription of several gene families and can reduce the extra-cellular pH. Different responses are induced by different auxin concentrations in different tissues, developmental stages, plant species, and so on.

Grind and find

The shoot apex is a major site for auxin biosynthesis. Auxin is synthesized through a variety of routes originating in the tryptophan biosynthetic pathway. Much of the auxin in plants is found conjugated to sugars or amino acids. Conjugates may act as degradation intermediates or as auxin stores. There is evidence for intracellular and extracellular sites for auxin perception. Several auxin binding proteins have been described but their *in vivo* roles are unclear.

Grow and show

Auxin is actively transported down the plant in a polar transport stream. Specific inhibitors of polar transport have established its role in controlling branching, elongation and tropic growth. The study of auxin-related mutants is improving our understanding of auxin biosynthesis, conjugation, uptake, efflux and signalling. For example, the *axr1* mutants pictured above (centre and right)

have reduced sensitivity to auxin. Their morphology confirms a role for auxin in branching, elongation and tropisms. The *AXR1* gene has been cloned, and suggests a model in which auxin signal transduction involves the conjugation of a ubiquitin-like molecule to target proteins.

Conclusions

The flow of auxin from shoot to root is a directional signal that can provide positional information, and information about the health of the shoot. Changes in flow, for example as a result of damage, can elicit appropriate responses in more basal tissues.

addition and measurement experiments suggests that changes in levels, transport and sensitivity are all important. This is perhaps hardly surprising given the number of variables that hormones appear to integrate. Regulation at all levels, from synthesis to final response, seems likely if hormone signalling systems are to integrate information from genotype, environment, physiological status, and developmental stage.

Recent progress

Recent years have seen dramatic advances in plant hormone biology, principally as a result of two factors. One is the rapid improvement in technologies for classical biochemical approaches. For example, detection of picogram quantities of auxin is now possible, providing solutions to some of the seemingly intractable problems of earlier years.

The second major advance has been the widespread adoption of *Arabidopsis* as a model system for plant molecular genetics. Mutants have been used for many years in hormone research, but because of lack of focus on a single system and the use of species not well suited for molecular genetic techniques, it was not possible to exploit the full power of the genetic approach. During the past decade, a large collection of *Arabidopsis* mutants with defects in hormone synthesis, conjugation, transport, perception and signal transduction have been isolated. These mutants have provided cloned genes for hormone receptors and transporters, as well as a variety of proteins involved in other aspects of hormone biology (see green box).

Although access to these cloned genes is obviously of great significance, the phenotypic analysis of the mutants has made an equally important contribution. The existence of isogenic lines which differ only in their ability to make, conjugate, transport, perceive or respond to particular hormones has established direct links between hormones and growth and development. Emerging themes include the importance of negative regulation. There are now several examples in which hormones act by relieving an inhibition, rather than directly promoting a response.

Armed with these new tools there is rapid progress in understanding how plant hormones act. It is abundantly clear that regulation occurs at multiple levels. Statements such as ‘auxin promotes cell division’ are essentially meaningless because it depends on which cell, where the auxin is coming from, whether there are rapid changes in auxin concentration, what the environmental conditions are, and the physiological status of the plant and the cell. We are finally beginning to get some answers and, as usual, it’s more complicated than we thought.

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Key references

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